On combustion 0.1628 and 0.2025 g. subs. yielded 0.2913 and 0.3659 g. CO_2 and 0.0863 and 0.1059 g. H_2O, corresponding to 48.8 and 49.2% C and 5.93 and 5.85% H. Calc. for lactose heptacetate ($C_{26}H_{36}O_{18}$): 49.3% C and 5.70% H.

An acetyl estimation was made by boiling two half-gram portions for four hours with 100 cc. $N/4$ H_2SO_4 in a quartz flask with a quartz reflux condenser, yielding 47.05 and 47.45% CH_3CO in comparison with the theoretical value, 47.36%.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE ILLINOIS WESLEYAN UNIVERSITY.]

THE QUANTITATIVE DETERMINATION OF MORPHINE IN THE VARIOUS ORGANS WHEN INJECTED INTO CATS AND RABBITS.

BY A. W. HOMBERGER AND J. C. MUNCH.

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During the past few years one of the authors has had submitted to him, at different times, organs from cadavers for the analysis of poisons—especially morphine and opium. In two cases, the suspects had been embalmed. In one case after seven days, and in the other after fifteen days, of interment, the liver, kidneys, stomach and spleen were brought for examination. The quantitative relations as well as the general chemical conduct of the alkaloids found in the different organs, raised the question as to whether this was due to the embalming fluid, or whether the length of time after burial were the cause of the fluctuating assimilation and distribution.

The following paper deals with a series of experiments carried out on cats and rabbits in order to find, if possible, what the effect of the embalming would be on the amount of alkaloid in the different organs in case death had resulted from alkaloid poisoning, to ascertain how much of the original product could be recovered under those conditions, and to find what organs might be the strongest assimilators of such alkaloids. The alkaloid used in this case was morphine.

The morphine used in these investigations was Merck & Company's "Morphinae Sulphas," U. S. P. VIII. Qualitative examination showed it to be free from foreign materials such as starch or other opium alkaloids. Quantitative determinations of the morphine present, by Mayer's Reagent, and determinations of the sulfuric acid present, as barium sulfate, showed that the morphine sulfate used agreed with the formula

$$(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O.$$

The desired amount of morphine sulfate was weighed out on an analytical balance, dissolved in distilled water and hypodermically injected into the mesenteric circulation. After a lapse of three hours, to allow for fixation in the organs of the body, the animals were chloroformed

and embalmed by cavity and arterial injection of a formaldehyde embalming fluid.

When the bodies were opened for analysis, the organs were always removed in the same order, first the stomach and intestines together, then, in order the spleen, liver, lungs, heart, kidneys, bladder and urine, and finally the brain. Then any parts wanted for qualitative analysis were cut out. Analyses were commenced at once to avoid any changes due to further decomposition.

Autenrieth's[1] modification of the Stas-Otto process was used, with several variations, for all the quantitative extraction of the organs. A longer time of extraction (five hours),[2] three extractions with ether in both acid and sodium hydroxide solution, and Puckner's technic of final extraction by hot amyl alcohol in slightly ammoniacal solution were used. The extracted morphine was dried at 60°, and calculated to its equivalent weight of morphine sulfate. Titrations of this residue agreed, within the limits of experimental error, with the gravimetric results obtained above. Qualitative tests of all residues were made to prove that the extractions were complete.

Dragendorff's[3] method was used for the analyses of both blood and urine. Puckner's[4] precautions were again followed. The extracted morphine was calculated to morphine sulfate, as in the Autenrieth extraction above. The weights in the following table are all expressed as morphine sulfate, $(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O$.

As may be noted in the table, the urine contains the largest single amount found in cats. The organs may be ranked according to morphine content, as the liver, kidneys, spleen and stomach. The urine content shows a great fluctuation, which is explained by Magnus[5] by the great variation in urinary secretion in animals, due to various factors such as quantity of food and water consumed, fecal excretion and animal idiosyncrasy. The appearance of morphine in the stomach of a cat following hypodermic mesenteric injection, is a matter of interest. Although only a small quantity is present, its constant appearance is similar to the case of arsenic, which, irrespective of the mode of introduction, always appears in the stomach. The constancy of results in the spleen extraction is important, the spleen of all organs, in both cats and rabbits, being subject to least variation in morphine content.

The results of extraction from rabbits are subject to less fluctuation during the course of the experiments, than with cats during the corresponding period. Arranged in order of morphine content, the results rank

[1] Autenrieth, "Detection of Poisons," **1915**, p. 57.

[2] THIS JOURNAL, **23**, 420 (1901).

[3] A. W. Blyth, "Poisons," **1906**, p. 313.

[4] THIS JOURNAL, **23**, 420 (1901).

[5] *Münch. Med. Woch.*, No. 28 (1906).

as kidneys, liver, urine, spleen and stomach. Rabbit "E" differed from the others, in that it received a larger dose of morphine, and was not embalmed. That the urine contains the greatest quantity of morphine agrees entirely with the results of Van Kaufmann-asser,[1] of Van Ryn,[2] and of Dorlencourt,[3] drawn from the analyses of rabbits killed with morphine.

TABLE I.

Data obtained from experimental results.

Morphine in the organs of cats, as gram morphine sulfate.

	Cat A. G.	Cat B. G.	Cat C. G.
Kidneys	0.040	0.032	0.027
Liver	0.055	0.057	0.042
Spleen	0.031	0.023	0.020
Stomach	0.005	0.002	0.005
Urine	0.064	0.031	0.043
Total found	0.195	0.145	0.137
Total injected	0.200	0.200	0.200
Percentage found	97.5%	72.5%	68.5%
Length of burial	Analysis started immediately after death	One month	Three months

TABLE II.

Morphine in the organs of rabbits, as gram morphine sulfate.

	Rabbit A. G.	Rabbit B. G.	Rabbit C. G.	Rabbit D. G.	Rabbit E. G.
Kidneys	0.0757	0.0654	0.0621	0.0473	0.0111
Liver	0.0601	0.0580	0.0574	0.0437	0.0214
Spleen	0.0122	0.0121	0.0120	0.0101	0.0343
Stomach	0.0060	0.0057	0.0052	0.0045	0.0072
Urine	0.0340	0.0304	0.0314	0.0311	0.0421
Total found	0.1889	0.1716	0.1681	0.1367	0.1161
Total injected	0.2000	0.2000	0.2000	0.2000	0.5000
Percentage found	94.5%	89.8%	84%	68.4%	23%
Length of burial	Analysis started immediately after death	2 weeks	4 weeks	6 weeks	8 weeks Unembalmed

Qualitative analyses of the tissues surrounding the organs examined giving uniformly negative results, osmosis can not account for the gradual decrease in morphine content. From the preceding results, it would seem that upon introducing morphine into the organs used in these anal-

[1] *Biochemische Ztg.*, **54**, 161.

[2] Van Ryn, *Chemisches Zentralblatt*, **1908**, [2] 174; *Pharmaceutisches Weekblad*, **44**, 1353 (1907).

[3] *Compt. rend.*, **56**, 1338.

yses, part of it combines with some cell constituent, forming a compound which is relatively more resistant to decomposition than is the uncombined morphine. Decomposition of the organ produces chemical compounds which break up the "free" morphine, but do not appreciably affect the "combined" morphine. This decomposition and removal of morphine produces the "preliminary drop" in morphine content observed.

The fairly constant results obtained between the close of this period and the beginning of the "secondary drop" represent the results of analyses made after the "free" morphine had been disintegrated, but before conditions were such that the "combined" morphine would be affected. This period of time is fairly constant for all the animals studied.

The gradual disintegration of the "combined" morphine, starting when conditions became favorable, would account for the "secondary" drop in morphine content. This decomposition would probably lead to complete removal from the body of all morphine as such.

This conclusion is best shown by the kidneys and liver, while the constant results for the spleen would seem to indicate that all the morphine deposited in that organ was present during decomposition as "combined" morphine, which began decomposition at the same time as the "combined" morphine of the other organs.

Summary.

From the preceding work the following conclusions may be drawn:

(1) By means of a five-hour extraction, and three extractions with ether in both acid and sodium hydroxide solution, Autenrieth's modification of the Stas-Otto process is capable of yielding 97.5% of the morphine injected into an animal, if analysis be started very soon after death.

(2) The presence of formaldehyde as embalming fluid, has no appreciable effect upon the extraction results.

(3) The loss of morphine from a cadaver proceeds in two stages, a preliminary drop due to the splitting up of the "free" morphine present, then a period during which the morphine content is fairly constant, followed by a secondary drop, due to the splitting up of the "combined" morphine, *i. e.*, that morphine which had combined with some cell constituent, thus escaping the preliminary decomposition.

(4) The "secondary" decomposition is greatly retarded by embalming, although there seems to be no effect on preliminary disappearance.

(5) Results of analyses of cats and rabbits which have received hypodermic injections of morphine, embalmed and subsequently analyzed, may be interpreted successfully by this theory of "combined morphine."

BLOOMINGTON, ILLINOIS.